SABRAO Journal of
Breeding & Genetics
31(1):17-22, 1999

INHERITANCE OF INDUCED DOMINANT AND RECESSIVE GENETIC MALE-STERILE MUTANTS IN RICE (*ORYZA SATIVA* L.)

X. ZHU[1] and J. N. RUTGER[2]

SUMMARY

Three genetic male steriles were selected from gamma-irradiated populations of southern US rice cultivars. Inheritance of these mutants was studied through progeny tests in M_3, M_4 or M_7 generations and through fertility characterization of pollen and spikelets at Stuttgart, Arkansas. For mutants Kaybonnet 1789 and Orion 1783, progenies from fertile plants in segregating rows were all fertile, and progenies of near-sterile plants segregated 1 fertile: 1 near-sterile, indicating that male sterility in each mutant was controlled by a single dominant gene. The other male sterile, Cypress 1819, was inherited as a single recessive gene, giving a good fit to a 3 fertile: 1 sterile ratio in nine segregating families. The two dominant male steriles showed partial pollen abortion, while the recessive mutant, Cypress 1819, showed complete pollen abortion. Seed set on steriles under open pollination was 32.9% for Kaybonnet 1789, 27.4% for Orion 1783, and 8.1% for Cypress 1819, while bagged seed set averaged 0.3, 3.5, and 0%, respectively. The usefulness of the dominant mutants for facilitating recurrent selection programs is discussed.

Key words: *Oryza sativa*, genetic male sterility, dominant, recessive, induced mutant, inheritance.

More than 70 genes for genetic male sterility and 35 combinations of cytoplasmic-genetic male sterility have been reported in rice (Kinoshita, 1997; Singh and Virmani, 1990). The wild abortive (WA) cytoplasmic-genetic male sterile has been very useful in hybrid rice production in China (Yuan et al., 1994). Among the genetic male steriles, photoperiod-sensitive genetic male steriles (pgms) and temperature-sensitive genetic male steriles (tgms) are being investigated for commercial production of hybrid rice, whereas the other classic male steriles have been proposed for facilitating crossing in population improvement schemes such as recurrent selection and backcross breeding in self-pollinating crops (Rutger and Shinjo, 1980; Sorrells and Fritz, 1982).

Male sterility in rice can be easily induced by irradiation (Rutger, 1992), and usually is controlled by one recessive gene. Although recessive male sterility can be used for crop population improvement, dominant male sterility is preferable, as sterility appears in every generation, versus only every second generation for recessives (Sorrells and Fritz, 1982). Dominant male steriles have been reported in cotton (Bowman and Weaver, 1979), wheat (Deng and Gao, 1982; Sasakuma et al., 1978), millet (Hu et al., 1986), and rice (Yan et al., 1989). This paper reports the discovery of two new dominant male steriles in rice induced by gamma-rays, as well as a typical recessive male sterile.

[1] China National Rice Research Institute, Hangzhou, Peoples Republic of China.
[2] National Rice Germplasm Evaluation and Enhancement Center, Stuttgart, AR, USA.
Corresponding Author. Fax: +1 870 673 7581; email: <jnrutger@futura.net>.

MATERIALS AND METHODS

Three male sterile mutants, induced in 1993 and 1994 by gamma-irradiation, were selected at Stuttgart, Arkansas, as individuals in the M_2 generations of three US cultivars: Kaybonnet (long grain type), Orion (medium grain type), and Cypress (long grain type). Generations were advanced in Arkansas summer nurseries and in Lajas, Puerto Rico winter nurseries. In those M_2 families showing apparent 3 fertile: 1 sterile segregations, the materials were propagated by growing progeny of fertile plants. In some families, apparent segregation ratios of 1 fertile: 1 near-sterile (hereafter referred to as sterile) were observed; those populations were propagated by growing progeny of both fertile and sterile plants. Detailed genetic analyses began in the M_5, M_6, or M_7 generations. The Orion mutant came from a 25 kR treatment; the other two came from 20 kR treatments. These materials were designated by their parental names and 1996 row numbers, viz., Kaybonnet 1789, Orion 1783, and Cypress 1819.

In 1996 in Arkansas, each hill was planted with the seeds of one panicle of a plant from the previous generation. The numbers of fertile and sterile panicles per hill were counted, and some panicles were selected for the 1997 progeny tests in Arkansas. On May 13-14, 1997, the seeds from each panicle were dry-sown in a 15-hill row 4.5 m long, with two or three seeds per hill. The rows were 0.3 m apart. Following permanent flooding one month later, each hill was thinned to one plant, and the extra seedlings were discarded or transplanted to vacancies between the hills. Thus, the final spacing between plants sometimes was 0.15 m, and almost all rows had more than 16 plants. As maturity approached, plants were scored as fertile if their panicles drooped and sterile if the panicles remained erect. In these populations, the difference between fertile and sterile plants was distinct, with fertile plants generally having seeds set in at least 80% of florets, and sterile plants setting 35 % or fewer seeds per floret, as was determined in subsequent seed set counts. Male sterile plants of each mutant, identified in the field by the shape and color of anthers, were examined for pollen fertility, as measured by I-KI staining. Sterile plants also were ratooned in the greenhouse or growth chamber for further pollen staining studies. Before anthesis, 8-10 spikelets which had opened that day, or would be opening on the next day, were collected and fixed in FAA solution [formalin (10 mL) + glacial acetic acid (5 mL) + 95% ethyl alcohol (50 mL) + water (35 mL)]. Four spikelets were randomly taken from the sample, then six anthers were squashed on a slide and observed for pollen stainability in a drop of 1% I-KI solution. Approximately 500 to 700 pollen grains of each mutant were observed. Each spikelet sample was examined in two fields at 200× enlargement with an Olympus BX50F microscope.

In each mutant, three panicle rows which were segregating for fertility were examined for seed set rates by sampling randomly one panicle per plant from both fertile and sterile plants. After harvest the seed set percentages were determined in all panicles of steriles and in random panicles of five fertiles per row. Five panicles of male sterile plants, recognized by the shape and color of anthers in the field, were bagged to determine the frequency of selfed seed setting.

To further confirm the segregation ratios, appropriate progeny tests were conducted in the 1997/98 Puerto Rico winter nursery, and in the 1998 Stuttgart nursery.

RESULTS

The numbers of fertile and sterile plants observed for the three mutants in the 1997 Arkansas nursery are shown in Table 1. For Kaybonnet 1789 and Orion 1783, the offspring of each fertile panicle were all fertile, except for two steriles among the 491 progeny plants of fertile panicles in Orion 1783, which may have been due to contamination during planting. The numbers of fertile and sterile plants in the progeny of the Kaybonnet 1789 sterile panicles closely fitted a 1 fertile: 1 sterile ratio, whereas the segregation ratio in the progeny of Orion 1783 approached 1:1, but showed a significant excess of sterile plants. A segregation ratio of 1 fertile: 1 sterile indicates the control of male sterility by a single dominant gene, so that *ms ms* plants are fertile and *Ms ms* plants are sterile. All pollen is *ms*, and hence open-pollinated steriles will segregate 1:1. The progeny of fertile panicles of Cypress 1819 selected from segregating hills, fitted a ratio of 1 all-fertile row: 2 rows segregating for fertility and sterility. Within the segregating rows, the numbers of fertile and sterile plants fitted a 3:1 ratio, showing that the male sterility of mutant Cypress1819 was controlled by a single recessive gene.

Table 1. Segregation for fertility and sterility in the pedigrees of three male sterile rice mutants.

Mutant name and test generations	No. of panicles sampled (1/plant)		No. of offspring Fertile	Sterile	Expected ratio	Goodness of fit χ^2
Kaybonnet 1789 M_5/M_6	Fertile	13	329	0		
	Sterile	32	398	393	1:1	$0.75 < P < 0.90$
Orion 1783 M_6/M_7	Fertile	18	489	2		
	Sterile	16	140	185	1:1	$0.01 < P < 0.025$
Cypress 1819 M_4/M_5	Fertiles from segregating families	12[1]	171	66	3:1	$0.25 < P < 0.50$

[1] Three families derived from M_4 panicles produced all fertiles in M_5 and nine families segregated, whereas the expected numbers were 4: 8. With Yates' correction, the goodness of fit $\chi^2 = 0.8$, $0.50 < P < 0.75$.

Male sterile plants in segregating populations of all three mutants could be recognized in the field by the shape and color of their anthers. The anthers of Kaybonnet 1789 and Orion 1783 were nearly normal in length and width, but the yellow coloration was a little whiter than fertile anthers. Cypress 1819 had tiny white anthers. The percentages of stainable pollen of the three mutants are shown in Table 2. Pollen staining of each mutant expressed similar trends in the three environments. Mean pollen staining of the original parents ranged from 83.9 to 87.8%. Mutants Kaybonnet 1789 and Orion 1783 had similar mean staining rates of 44.1 and 47.8%, respectively. Although definitive studies remain to be done, it is the general observation of the authors that rice anthers with only half stainable pollen do not dehisce as readily as normal anthers. Pollen staining of Cypress 1819 was only 2.2%. Using the criteria of Lu and Rutger (1984), mutants

Kaybonnet 1789 and Orion 1783 were classified into the partial pollen abortion (PPA) type, and Cypress 1819 belonged to the complete pollen abortion (CPA) type.

Table 2. Stainability of the pollen of single plants of three male sterile mutants and their parents sampled from the field, greenhouse and growth chamber.

Mutants and parents	Percent pollen staining			
	Field	Greenhouse	Growth chamber	Mean[1]
Kaybonnet		87.4	88.2	87.8
Kaybonnet 1789	44.7	40.6	47.1	44.1
Orion		82.3	91.4	86.8
Orion 1783	46.5	41.1	55.9	47.8
Cypress		85.7	82.2	83.9
Cypress 1819	2.5	3.8	0.4	2.2

[1] For greenhouse and growth chamber data for the parents; for all environments for the mutants.

The mean seed set rates of the parents and fertile and sterile segregants of each mutant are shown in Table 3. Parent seed sets ranged from 89.0 to 95.4%. The fertile segregants of the mutants had seed set rates of 81.9 to 89.9%, which was similar to, or approached, the parental rates. The sterile segregants set seed at rates notably less than parents and fertile groups, particularly for Cypress 1819. Bagged selfing rates of the three mutants were considerably lower than the open-pollinated rates.

Table 3. Percentage of spikelets setting seed in three male sterile mutants and their parents in the field.

Mutants and parents	Fertility type	Mean percent seed set	
		Unbagged	Bagged
Kaybonnet	fertile	95.4 ± 2.32 (3)[1]	
Kaybonnet 1789	fertile	89.9 ± 2.93 (15)	
	sterile	32.9 ± 4.08 (33)	0.3 (5)
Orion	fertile	91.5 ± 4.53 (3)	
Orion 1783	fertile	81.9 ± 7.04 (15)	
	sterile	27.4 ± 3.04 (30)	3.5 (5)
Cypress	fertile	89.0 ± 2.67 (3)	
Cypress 1819	fertile	88.9 ± 0.65 (15)	
	sterile	8.1 ± 0.96 (17)	0 (5)

[1] Number of panicles examined.

In the 1997/98 Puerto Rico progeny tests of five open-pollinated, sterile Kaybonnet 1789 plants from the 1997/98 Stuttgart segregating M_6 population, all families gave 1 fertile: 1 sterile segregations. The progeny of 25 fertile and 29 sterile plants from the M_7 Puerto Rico rows were grown in the 1998 Stuttgart nursery, where the fertile plants

gave only fertile progeny, and the sterile plants segregated 1:1. A census sample of five M_8 segregating rows gave 36 fertile: 39 sterile plants, a satisfactory fit ($0.50 < P < 0.75$) to the expected 1:1 ratio for dominant male sterility.

All four of the M_8 Orion 1783 families grown in Puerto Rico in 1997/98 from seed of sterile M_7 plants gave apparent 1 fertile: 1 sterile segregations. Open-pollinated seeds from 11 fertile and 12 sterile M_8 plants were grown in the 1998 Stuttgart nursery, where the offspring of fertile plants were all fertile, and the offspring of sterile plants segregated in 1:1 ratios. A census sample of 6 segregating M_9 rows gave 31 fertile: 35 sterile plants, which satisfactorily fitted ($0.50 < P < 0.75$) the expected 1:1 dominant male sterile ratio.

Five of the fertile M_5 plants of Cypress 1819 in the 1997 Stuttgart nursery gave progeny which segregated 3 fertile: 1 sterile plants in the M_6 generation. Among the 40 selfed families from fertile plants in segregating M_6 families, the observed segregation was 12 all-fertile: 28 segregating families, a satisfactory fit ($0.50 < P < 0.75$) to the ratio of 1 fertile: 2 segregating families expected of a recessive male sterile.

DISCUSSION

Most importantly, the present study documented the discovery of two dominant genetic male steriles. This is only the second report of dominant male sterility in rice. The rarity of dominant male sterility is in contrast to the frequent occurrence of recessive male sterility. Until appropriate allelism tests are done, it is not known if these two mutants are different from each other and from the previously reported dominant mutant. The excess of steriles in the open-pollinated progeny of M_6 and M_8 male sterile plants of Orion 1783 may be due to the greater viability of *Ms* eggs, or perhaps to a low level of self-pollination. No dehiscence of the anthers of either Kaybonnet 1789 or Orion 1783 was observed, but a low level may have remained undetected.

Dominant male steriles are especially interesting because they are much more useful than recessive male steriles for expediting recurrent selection schemes. Dominant male sterility reappears in the first generation after crossing, whereas recessive male sterility does not reappear until the second generation. The two dominant male steriles in the present study, Kaybonnet 1789 and Orion 1783, also showed considerable unbagged seed set, indicating that outcrossing occurred readily in these two sources. Therefore, both mutants would be suitable for generating high rates of outcrossing and recombination in large scale population improvement programs.

A classical recessive male sterile, Cypress 1819, also was reported in this study, primarily as a reference type. During the course of these investigations, many apparent recessive male steriles were induced but their inheritance was not pursued because numerous recessive male steriles of rice were already available from previous studies (Kinoshita, 1997; Rutger, 1992).

REFERENCES

Bowman, D. T., and J. B. Weaver, Jr. 1979. Analyses of a dominant male-sterile character in upland cotton. II. Genetic studies. *Crop Sci.* 19:628-630.

Deng, J., and Z. Gao. 1982. Discovery and determination of a dominant male-sterile gene and its importance in genetics and wheat breeding. *Scientia Sinica (Series B)* 25:508-516.

Hu, H., S. Ma, and Y. Shi. 1986. The discovery of a dominant male-sterile gene in millet (*Setaria italica*). *Acta Agron. Sinica* 12(2):73-78 (In Chinese with English summary).

Kinoshita, T. 1997. Report of committee on gene symbolization. Gene symbols and information on male sterility. *Rice Genet. Newsl.* 14:13-22.

Lu, Y. G., and J. N. Rutger. 1984. Anther and pollen characteristics of induced genetic male sterile mutants in rice (*Oryza sativa* L.). *Envir. Exp. Bot.* 24:209-218.

Rutger, J. N. 1992. Impact of mutation breeding in rice - a review. *Mutation Breed. Rev.* 8: 1-23. FAO/IAEA, Vienna, Austria.

Rutger, J. N., and C. Shinjo. 1980. Male sterility in rice and its potential use in breeding. *In: Innovative Approaches to Rice Breeding*. Selected Papers from the 1979 International Rice Research Conference. Int.Rice Res. Inst., Los Baños, Philippines. pp. 53-66.

Sasakuma, T., S. S. Maan, and N. D. Williams. 1978. EMS-induced male-sterile mutants in euplasmic and alloplasmic common wheat. *Crop Sci.* 18:850-853.

Singh, R. B., and S. S. Virmani. 1990. Report of progress in the technology and development of hybrid rice in Asia. *FAO Int. Rice Commiss. Newsl.* 39:133-145.

Sorrells, M. E., and S. E. Fritz. 1982. Application of a dominant male-sterile allele to the improvement of self-pollinated crops. *Crop Sci.* 22: 1033-1035.

Yan, L. J. Zhang, C. Zhu, Y. Ou, J. Li, and Y. Cai. 1989. The preliminary evaluation of a dominant male-sterile gene in rice. *Acta Agron. Sinica* 15:174-180 (In Chinese; English summary).

Yuan, L. P., Z. Y. Yang, and J. B. Yang. 1994. Hybrid rice research in China. *In*: Virmani, S. S., ed., *Hybrid rice technology: new developments and future prospects*. Int. Rice Res. Inst., Manila 1099, Philippines. pp. 143-147.